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Influence of elevated CO_2 and mild water stress on nonstructural carbohydrates in field-grown cotton tissues

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Abstract

Root, stem and leaf tissues, from cotton plants exposed to CO_2 at ambient (370 μmol mol^{-1} (control)) or elevated (550 μmol mol^{-1} (FACE; free-air carbon dioxide enrichment)) levels in the field during the 1990 and 1991 growing seasons, were analyzed for nonstructural carbohydrates (glucose, fructose, sucrose and starch). Besides the FACE treatment, these plants were also exposed to two irrigation levels: 100% and 67% replacement of evapotranspiration. FACE had a greater effect upon cotton plant nonstructural carbohydrates than did irrigation treatments. Leaf carbohydrate content was increased by FACE, but this increase was much more pronounced in the stems and roots. Starch and soluble sugars in leaves in FACE plots tended to be consistently greater than in control leaves, without much change in carbohydrate content during the growing season. In contrast, root and stem, starch and soluble sugar pools were strongly increased by FACE and fluctuated strongly during the growing season. In both seasons, stem and taproot nonstructural carbohydrate content passed through a minimum during periods of heavy boll set. The fluctuations in stem and root carbohydrate content were therefore probably caused by the varying metabolic demands of the developing plant. These results suggest that a significant effect of CO_2 enrichment on starch-accumulating plants is an increase of nonstructural carbohydrate, especially starch, in nonleaf storage pools. This buildup occurs somewhat independently of the water status of the plant, and these enlarged pools can be drawn upon by the growing plant to maintain growth during periods of high metabolic demand.

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1. Introduction

Many species of plants respond to elevated CO_2 by increasing their leaf starch content. Mauney et al. (1979) found that cotton, soybean, sunflower and sorghum leaves all responded to elevated CO_2 by increasing their starch content, but among these species elevated CO_2 increased starch in cotton leaves to the greatest extent. Some plant species, particularly those grown at lower temperatures, exhibit little response in biomass to elevated aerial CO_2 (Tissue and Oechel, 1987; Grulke et al., 1990). As a rule, crops such as cotton, which are grown in a subtropical environment, respond positively to elevated CO_2 by increasing their biomass to a greater extent than those grown at ambient CO_2 (Idso et al., 1987; Hogan et al., 1991). This biomass increase is generally thought to be due to an increase in photosynthetic carbon fixation, although a depression of respiration by elevated CO_2 may also be involved (Idso and Kimball, 1992). Carbohydrate production beyond the metabolic requirements of leaf metabolism and carbon export leads to leaf starch accumulation in many plant species, including cotton. At the end of an illuminated period, sucrose in cotton leaf cells is depleted and leaf starch is slowly converted to sucrose for export to the rest of the plant (Hendrix and Grange, 1991). High metabolic demand for leaf sucrose, water stress or poor photosynthetic conditions can slow the rate of accumulation of starch in cotton leaves during illuminated periods. Preliminary experimentation (Hendrix, 1992) showed that free-air CO_2 enrichment (FACE) caused a significant increase in cotton leaf starch over that in ambient conditions and that this increase was a function of the time in the growing season. The present experiments were conducted to determine if pools of soluble sugars and starch in cotton taproot and stem were also altered over a growing season in cotton plants exposed to FACE and what effect mild water stress might have on these pool alterations.

2. Materials and methods

Cotton (*Gossypium hirsutum* L. cv. 'Deltapine 77') was planted in a field on the University of Arizona agricultural center at Maricopa, Arizona, in April 1990 and 1991. Crop management and culture have been described by Mauney et al. (1994). The plots were exposed to two atmospheric CO_2 concentrations (370 μmol mol^{-1} CO_2 (control) and 550 μmol mol^{-1} CO_2 (FACE)) and two water regimes (100% ('wet') and 67% ('dry') replacement of evapotranspiration). Samples were collected from each of the eight experimental plots in the field, four of which were control plots and four of which were FACE plots. Each of these plots was further subdivided into a wet and a dry subplot (Mauney et al., 1994). Irrigation and fertilization were applied to the plants by a subsurface drip irrigation system; irrigation of the crop was initiated on 20 May in both 1990 and 1991.

2.1. Sample collection

At each sampling, leaf tissue was collected from six of the most recently fully

expanded leaves from separate plants in each of the 16 subplots. Leaves in each subplot were sampled at approximately monthly intervals beginning on 2 July 1990 and 18 June 1991 by removing six leaf discs, each about 45 mm^2 in area, from each leaf sampled. The discs were punched out of the leaves in a regular pattern, close to the margin and between the major veins. After removal from the plant, they were quickly transferred to test tubes containing 2 ml of ice-cold 80% aqueous ethanol. The tubes were transported to the laboratory in crushed ice and stored at −19°C until they were analyzed for sugars (Hendrix and Peelen, 1987; Hendrix, 1993). At 2-week intervals during the season approximately 10 plants were harvested from the same subplots, their leaves removed and their areas calculated using a Li-Cor (Lincoln, NE, USA) model 3100 area meter (Mauney et al., 1994). The numbers of green and open bolls per plant were noted after harvest, and the total weight of the various plant parts was determined after drying to uniform weight in a hot air environment. Small pieces of dried taproot and stem were removed from the dried plants and sectioned radially at 200 μm intervals using a hand microtome. These sections were weighed and analyzed for starch by the same procedure as that used for the leaf discs.

2.2. Analysis of nonstructural carbohydrates

Soluble sugars (glucose, fructose and sucrose) were removed from plant samples by extraction with hot (80°C) 80% ethanol. After bringing the ethanol washes to a known volume, an aliquot was removed from each sample and treated with activated charcoal to remove substances which interfered with subsequent sugar analyses (Hendrix and Peelen, 1987). Starch in the ethanol-insoluble residue was gelatinized by heating in 0.2 M KOH for 1 h. After this treatment, the KOH was neutralized with acetic acid and the starch converted to glucose by adding aliquots of α-amylase (EC 3.2.1.1) and amyloglucosidase (EC 3.2.1.3) to each tube (Hendrix, 1993).

Glucose in these extracts was then determined by a microplate colorimetric analysis procedure which utilized glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and the chromophore iodonitrotetrazolium violet. The enzymes phosphoglucose isomerase (EC 5.3.1.9) and invertase (EC 3.2.1.26) were sequentially added to the glucose detection mixture, to detect fructose and sucrose in the alcoholic extracts (Hendrix and Peelen, 1987; Hendrix, 1993).

Leaf areas and dry weight of various tissues were determined for plants removed from the field at various times during the season. Curvilinear relationships were determined between these values and the amount of carbohydrate per unit of field area using SigmaPlot computer graphics software (Jandel Scientific, San Rafael, CA, USA) using a planting density of 10 plants m^{-2} (Mauney et al., 1994) and a row spacing of 1 m.

3. Results and discussion

Significant differences in crop development occurred in these cotton fields during the 1990 and 1991 growing seasons (Figs. 1 and 2). In all treatments, the plants

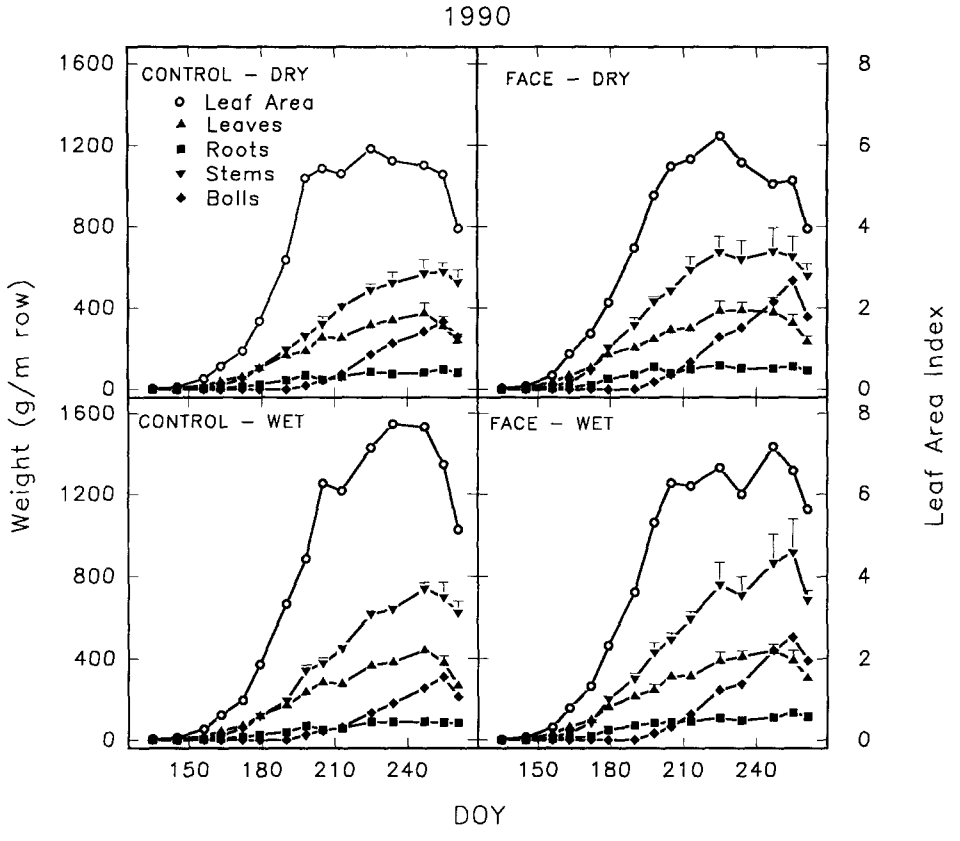

Fig. 1. Cotton plant phenology and leaf area index (crop leaf area per unit of field ground area) during the 1990 growing season in ambient and elevated CO_2. DOY stands for day of year. Data plotted in this and subsequent figures represent means ±SE of six replicate samples.

produced approximately twice the leaf area and total mass in 1990 than in 1991. A partial explanation of this difference might be the difference in weather between these two seasons; record high temperatures were recorded throughout the 1990 growing season, which was markedly warmer than that of 1991. In both years, FACE caused a significant increase in total plant and boll weight but not in leaf area index (Figs. 1 and 2). The wet treatment appeared to have little effect upon leaf area in 1990, but caused a slight increase in 1991, especially in the control plots. The 1990 crop appeared much more lush and prone to lodging than that of 1991 and, at certain times during the year, the plants in the 1990 crop within the FACE rings had upper leaves which exhibited a violet coloration, suggestive of high flavonoid content. Increased flavonoid content in plant leaf tissues often accompanies elevated levels of leaf sugars (Harborne, 1980), as was found in the leaves from the FACE rings during these periods (Fig. 3).

In irrigated cotton grown in the southwest of the USA, plants typically set numerous bolls during mid-June to mid-July; flowering and boll set then typically

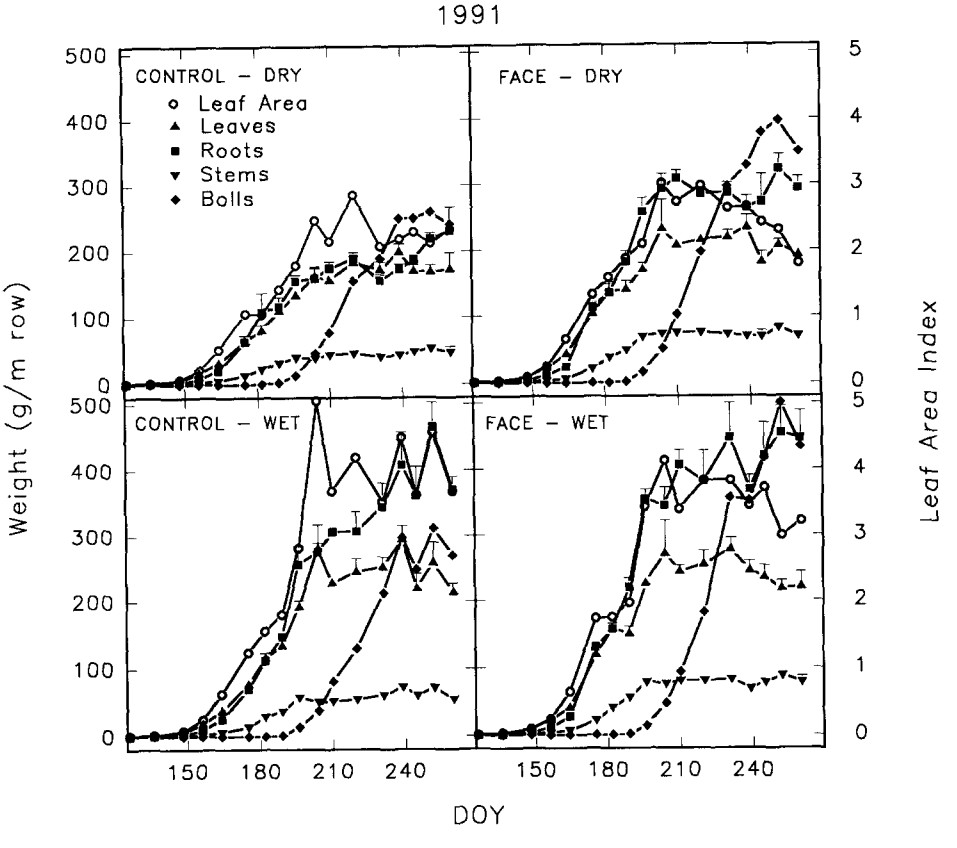

Fig. 2. Cotton plant phenology and leaf area index during the 1991 growing season in ambient and elevated CO_2. All details as for Fig. 1.

slows markedly during mid-July to mid-August (see Mauney et al., 1994). This cessation of flowering as a result of heavy boll load is referred to as 'cutout'. In both the 1990 and 1991 growing seasons, cutout occurred during August (Figs. 1 and 2; Mauney et al., 1994). Leaf sugars and starch per meter of crop row in all plots increased until cutout in August and then diminished somewhat toward the end of the growing season (Fig. 3). In both years, the FACE treatment caused the leaf starch content to be significantly increased over that in control leaves. As the FACE treatment was initiated in mid-season in 1990 (Fig. 3), the pronounced rise in leaf starch after the start of the CO_2 fumigation might be a response of the leaves to the additional CO_2. A similar burst in photosynthesis and leaf starch concentration was noted in greenhouse experiments with cotton (Mauney et al., 1979). Soluble sugars exhibited a similar pattern, but it was not as pronounced as for leaf starch. Leaves from the dry control plots consistently had the lowest nonstructural carbohydrate concentration and, before cutout, leaves from the wet controls had a lower soluble sugar content than those from either of the FACE treatments.

The nonstructural carbohydrates in cotton leaves are metabolic pools which reflect

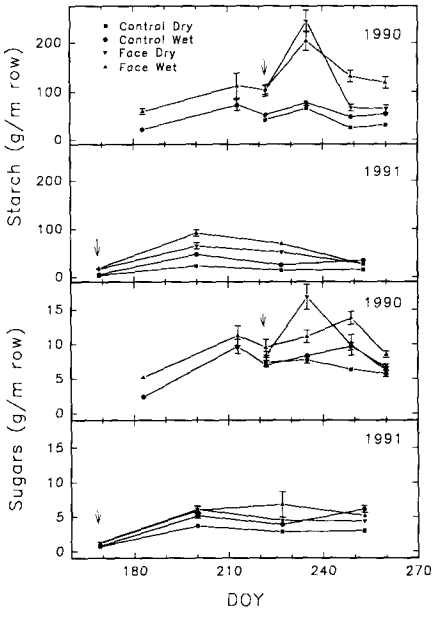

Fig. 3. Soluble sugars (glucose–fructose–sucrose) and starch in cotton leaves exposed to two levels of CO_2 and water stress. All samples were collected at approximately 14:00 h. Vertical arrows mark the time when leaf sugar and starch measurements in the FACE treatment was begun.

the amount of carbon entering the plant. Both leaf starch and leaf soluble carbohydrate pools turn over relatively rapidly, and the size of these pools at any time is a product of the formation of sugars through photosynthesis and their utilization by growing points throughout the plant. The relationship between leaf pool sizes in plant leaves and developmental stage or environmental factors depends upon the species of plant involved. Cotton leaves, which export carbon to the rest of the plant as sucrose (Tarczynski et al., 1992), increase their sucrose pools immediately upon being illuminated in the morning (Hendrix and Huber, 1986; Hendrix and Grange, 1991). If the metabolic demands upon the leaf sucrose pool (assimilation within the leaf and leaf export) are met, excess photosynthate produced during an illuminated period is stored within leaf chloroplasts as starch. Starch accumulated during these periods is converted to sucrose during dark periods for export as sucrose; if sufficient starch does not accumulate in cotton leaves, carbon export rates at night can be lowered or even cease (Hendrix and Grange, 1991). This is particularly important in cotton, as it is a plant in which a number of important processes, such as boll and stem expansion and, commonly, leaf growth, occur during dark periods (Radin, 1983). Exposure of cotton leaves to elevated CO_2 during daylight hours increased leaf starch content and therefore increased the ability of the leaves of these plants to continue to export carbon at night during periods of cloudy weather, water stress or heavy boll load, when strong metabolic demands were placed upon leaf carbohydrate pools.

The starch and soluble sugar content of cotton stems and taproots had quanti-

tatively similar seasonal patterns during both growing seasons (Figs. 4 and 5). The FACE treatments had a much greater effect upon the nonstructural carbohydrates in cotton root and stem tissues than did the water treatments. In all treatments the total amount of starch in cotton stems exceeded that in the taproots. Stem starch pools also exhibited strong fluctuations during both growing seasons, and the pattern of these fluctuations was not significantly altered by the water treatments. The starch pool in cotton roots and stems appears to be a metabolic reserve which is drawn upon during periods of great metabolic demand. Developing cotton fruits (bolls) are very strong carbohydrate sinks. The epidermal hairs (cotton fibers) on ovules within these bolls increase in dry weight as rapidly as 15% per day (Schubert et al., 1986), and these fibers are almost entirely made up of cellulose. As the efficiency of conversion of incoming photosynthate by the boll to dry matter varies from 33 to 54% (Baker et al., 1972), approximately 30% of the dry weight of the fibers must be imported to these developing fruits each day during their period of most rapid fiber elongation. For a cotton plant with a heavy fruit load, this can mean that more carbohydrate moves to maturing bolls (i.e. reproductive growth) each day than is created in photosynthesis. Rapid root or stem (i.e. vegetative) growth can also stress the ability of

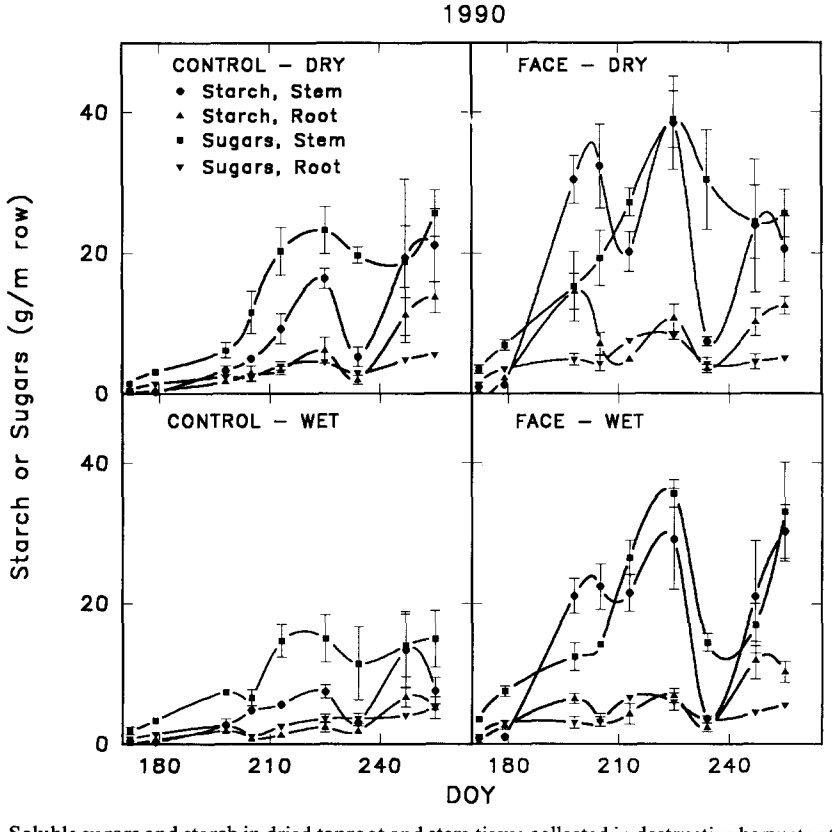

Fig. 4. Soluble sugars and starch in dried taproot and stem tissue collected in destructive harvests at various times during the 1990 growing season.

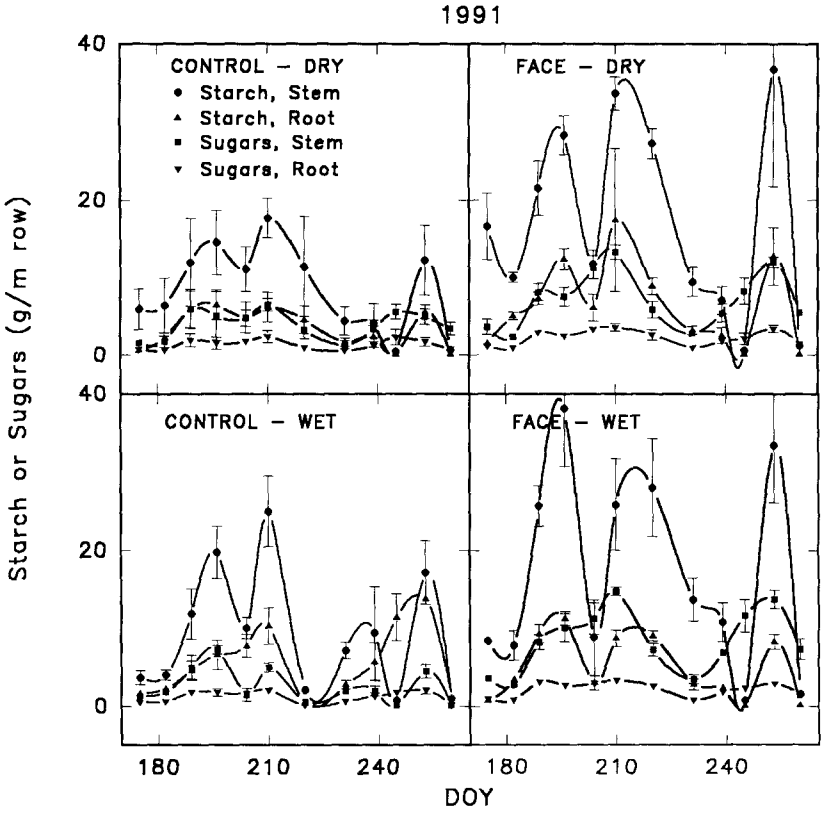

Fig. 5. Soluble sugars and starch in dried taproot and stem tissue collected in destructive harvests at various times during the 1991 growing season.

cotton leaves to supply sufficient carbohydrate to growth points. As a result, strong changes in vegetative and reproductive growth occur at various times during the growing season in irrigated cotton. Cotton roots must grow rapidly to reach sufficient water to supply the ever-expanding leaf canopy. Likewise, rapid stem growth occurs before and after cutout. The cotton plant responds to the carbohydrate demand of heavy boll set in two ways: (1) it stops vegetative growth and flowering, i.e. it undergoes cutout (discussed above); (2) it calls upon metabolic reserves such as the starch in stem and taproot tissue. Plants exposed to FACE might therefore have an advantage over those grown at ambient CO_2 levels during these periods, as the additional starch in their stems and roots might allow them to continue growth during periods when plants with lesser reserves have entered cutout. In fact, delays in the timing of cotton plant cutout in cotton plants exposed to FACE, compared with those exposed to ambient CO_2 levels, has been observed (Mauney et al., 1992).

A major difference between the plants grown during the 1990 and 1991 seasons is that the stem sugar (i.e. glucose–fructose–sucrose) content in all four treatments was considerably greater in 1990 than in 1991. In many instances during 1990, the stem soluble sugar content exceeded the stem starch content; this was especially true in the

control treatments regardless of irrigation. In all treatments during both growing seasons the soluble sugar content of the taproots was relatively low. Although starch in the roots is less abundant than stem starch, it also exhibits seasonal pool fluctuations. In many cases, these fluctuations occur at the same time as the stem starch pool size changes, suggesting that starch in both compartments is added to or drawn down at the same time during the season. This observation appears reasonable, as cotton taproots and stems are contiguous organs with very similar anatomy.

In starch-accumulating plants such as cotton, increasing atmospheric CO_2 appears to cause a significant increase in nonleaf carbohydrate pools. At least for mild water stress, this accumulation appears to be independent of plant water status. These pools can be drawn upon during periods of high metabolic demand, such as heavy fruit set or root growth, to cause such plants to be able to resist metabolically stressful periods better than plants grown in ambient CO_2.

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